

WHAT IS CLAIMED IS:

Selection of D-able subsites

1 1. A method of selecting a target site within a target sequence for
2 targeting by a zinc finger protein comprising:
3 providing a target nucleic acid to be targeted by a zinc finger protein;
4 outputting a target site within the target nucleic acid comprising 5'NNx
5 aNy bNzc3', wherein

6 each of (x, a), (y, b) and (z, c) is (N, N) or (G, K);
7 at least one of (x, a), (y, b) and (z, c) is (G, K). and
8 N and K are IUPAC-IUB ambiguity codes.

1 2. The method of claim 1, further comprising selecting a plurality of
2 potential target sites within the target nucleic acid and outputting a subset of the plurality
3 of potential target segments comprising 5'NNx aNy bNzc3', wherein

4 each of (x, a), (y, b) and (z, c) is (N, N) or (G, K);
5 at least one of (x, a), (y, b) and (z, c) is (G, K). and
6 N and K are IUPAC-IUB ambiguity codes.

1 3. The method of claim 2, wherein the target nucleic acid comprises a
2 target gene.

1 4. The method of claim 1, wherein at least two of (x, a), (y, b) and (z,
2 c) is (G, K).

1 5. The method of claim 1, wherein all three of (x, a), (y, b) and (z, c)
2 are (G, K).

1 6. The method of claim 1, wherein the zinc finger protein comprises
2 three fingers.

1 7. The method of claim 1, wherein the target site comprises first and
2 second target segments, each comprising 5'NNx aNy bNzc3', and the method further
3 comprises selecting the second target segment.

1 8. The method of claim 7, wherein in the second segment at least two
2 of (x, a), (y, b) and (z, c) are (G, K).

1 9. The method of claim 8, wherein in the second segment all three of
2 (x, a), (y, b) and (z, c) are (G, K).

1 10. The method of claim 10, wherein the first and second target
2 segment are separated by fewer than 5 bases in the target site.

1 11. The method of claim 10, wherein the first target segment comprises
2 5'NNN NNN NNG3', the second target segment comprises 5'KNx aNY bNzc3' and
3 there are zero bases separating the first and second target segments in the target site.

1 12. The method of claim 7, further comprising synthesizing step
2 comprises synthesizing a first zinc finger protein comprising three zinc fingers that
3 respectively bind to the NNx aNy and bNz triplets in the target segment and a second
4 three fingers that respectively bind to the NNx aNy and bNz triplets in the second target
5 segment.

1 13. The method of claim 1, further comprising synthesizing a zinc
2 finger protein comprising first, second and third fingers that bind to the bNz aNy and
3 NNx triplets respectively.

1 14. The method of claim 13, wherein each of the first, second and third
2 fingers is selected or designed independently.

1 15. The method of claim 13, wherein a finger is designed from a database
2 containing designation of zinc finger proteins, subdesignations of finger components, and
3 nucleic acid sequences bound by the zinc finger proteins.

1 16. The method of claim 13, wherein a finger is selected by screening
2 variants of a zinc finger binding protein for specific binding to the target site to
3 identify a variant that binds to the target site.

4 17. The method of claim 13, further comprising contacting a sample
5 containing the target nucleic acid with the zinc finger protein, whereby the zinc finger
6 protein binds to the target site revealing the presence of the target nucleic acid or a
7 particular allelic form thereof.

1 18. The method of claim 13, further comprising contacting a sample
2 containing the target nucleic acid with the zinc finger protein, whereby the zinc finger
3 protein binds to the target site thereby modulating expression of the target nucleic acid

1 19. The method of claim 1, wherein the target site occurs in a coding
2 region of a gene

1 20. The method of claim 1, wherein the target site occurs within or
2 proximal to a promoter, enhancer, or transcription start sit

1 21. The method of claim 1, wherein the target site occurs outside a
2 promoter, regulatory sequence or transcriptional start site within the target nucleic acid.

Selection of Target Sites Using a Correspondence Regim

1 22. A method for selecting a target site within a polynucleotide for
2 targeting by a zinc finger protein, comprising:
3 providing a polynucleotide sequence;
4 selecting a potential target site of within the polynucleotide
5 sequence; the potential target site comprising contiguous first, second and third triplets of
6 bases at first, second and third positions in the potential target site;
7 determining a plurality of subscores by applying a correspondence regime
8 between triplets and triplet position in a sequence of three contiguous triplets, wherein
9 each triplet has first, second and third corresponding positions, and each combination of
10 triplet and triplet position has a particular subscore

11 calculating a score for the potential target site by combining subscores for
12 the first, second, and third triplets;

13 repeating the selecting, determining and calculating steps at least once on a
14 further potential target site comprising first, second and third triplets at first, second and
15 third positions of the further potential target site to determine a further score;

16 providing output of at least one potential target site with its score.

1 23. The method of claim 22, wherein output is provided of the
2 potential target site with the highest score.

1 24. The method of claim 22, wherein output is provided of the n
2 potential target sites with the highest scores, and the method further comprises providing
3 user input of a value for n.

1 25. The method of claim 22, wherein the subscores are combined by
2 forming the product of the subscores.

1 26. The method of claim 25, wherein the correspondence regime
2 comprises 64 triplets, each having first, second, and third corresponding positions, and
3 192 subscores.

1 27. The method of claim 22, wherein the subscores in the
2 correspondence regime are determined by assigning a first value as the subscore of a
3 subset of triplets and corresponding positions, for each of which there is an existing zinc
4 finger protein that comprising a finger that specifically binds to the triplet from the same
5 position in the existing zinc finger protein as the corresponding position of the triplet in
6 the correspondence regime; assigning a second value as the subscore of a subset of
7 triplets and corresponding positions, for each of which there is an existing zinc finger
8 protein that comprises a finger that specifically binds to the triplet from a different
9 position in the existing zinc finger protein than the corresponding position of the triplet in
10 the correspondence regime; and assigning a third value as the subscore of a subset of
11 triplets and corresponding positions for which there is no known zinc protein comprising
12 a finger that specifically binds to the triplet.

1 28. The method of claim 22, wherein the correspondence regime is
2 shown in Table 1.

4 wherein each of (x, a), (y, b) and (z, c) is (N, N) or (G, K);

5 at least one of (x, a) , (y, b) and (z, c) is (G, K) . and

6 N and K are IUPAC-IUB ambiguity codes.

1 34. The method of claim 22, wherein output is provided of at least a
2 nonoverlapping pair of potential target sites and their scores, the members of the pair
3 being separated by five or fewer bases in the polynucleotide.

Design of ZFPs using a Database

35. A method of producing a zinc finger protein comprising:

2 (a) providing a database comprising designations for a plurality of zinc
3 finger proteins, each protein comprising at least first, second and third fingers, and
4 subdesignations for each of the three fingers of each of the zinc finger proteins;

5 a corresponding nucleic acid sequence for each zinc finger protein, each
6 sequence comprising at least first, second and third triplets specifically bound by the at
7 least first, second and third fingers respectively in each zinc finger protein, the first,
8 second and third triplets being arranged in the nucleic acid sequence (3'-5') in the same
9 respective order as the first, second and third fingers are arranged in the zinc finger
10 protein (N-terminal to C-terminal);

11 (b) providing a target site for design of a zinc finger protein, the target site
12 comprising continuous first, second and third triplets in a 3'-5' order,

13 (c) for the first, second and third triplet in the target site, identifying first,
14 second and third sets of zinc finger protein(s) in the database, the first set comprising zinc
15 finger protein(s) comprising a finger specifically binding to the first triplet in the target
16 site, the second set comprising zinc finger protein(s) comprising a finger specifically
17 binding to the second triplet in the target site, the third set comprising zinc finger
18 protein(s) comprising a finger specifically binding to the third triplet in the target site;

(d) outputting designations and subdesignations of the zinc finger proteins in the first, second, and third sets identified in step (c).

1 36. The method of claim 35, further comprising:

2 (e) producing a zinc finger protein that binds to the target site comprising
3 a first finger from a zinc finger protein from the first set, a second finger from a zinc
4 finger protein from the second set, and a third finger from a zinc finger protein from the
5 third set.

1 37. The method of claim 36 further comprising identifying subsets of
2 the first, second and third sets, the subset of the first set comprising zinc finger protein(s)
3 comprising a finger that specifically binds to the first triplet in the target site from the
4 first finger position of a zinc finger protein in the database; the subset of the second set
5 comprising zinc finger protein(s) comprising a finger that specifically binds to the second
6 triplet in the target site from the second finger position in a zinc finger protein in the
7 database; the subset of the third set comprising zinc finger protein(s) comprising a finger

8 that specifically binds to the third triplet in the target site from a third finger position in a
9 zinc finger protein in the database;

10 wherein

11 the outputting step comprising outputting designations and
12 subdesignations of the subset of the first, second and third sets; and

13 the producing step comprising producing a zinc finger protein comprising
14 a first finger from the first subset, a second finger from the second subset, and a third
15 finger from the third subset.

1 38. The method of claim 37, wherein the outputting comprises
2 outputting the designations and subdesignations of the subsets of the first, second and
3 third sets, and the first, second and third sets minus their respective subsets.

1 39. The method of claim 38, wherein each of the subsets is a null set.

1 40. The method of claim 35, wherein the target site is provided by user
2 input.

1 41. The method of claim 35 wherein the target site is provided by the
2 method of claim 1 or claim 22.

1 42. A method of producing a zinc finger protein comprising:

2 (a) providing a database comprising
3 designations for a plurality of zinc finger proteins, each
4 protein comprising at least first, and second fingers,
5 subdesignations for each of the fingers of each of the zinc
6 finger proteins; and

7 a corresponding nucleic acid sequence for each zinc finger protein, each
8 sequence comprising first and second triplets specifically bound by the first and second
9 fingers respectively, the triplets being arranged in the nucleic acid sequence (3'-5') in the
10 same respective order as the first and second fingers are arranged in the zinc finger
11 protein (N-terminal to C-terminal);

12 (b) providing a target site for design of a zinc finger protein, the
13 target site comprising contiguous first, and second triplets ordered 3'5' in the target site;

14 (c) for the first and second triplet in the target site, identifying first
15 and second sets of zinc finger protein(s) in the database, the first set comprising zinc
16 finger protein(s) comprising a finger specifically binding to the first triplet in the target
17 site, the second set comprising zinc finger protein comprising a finger specifically
18 binding to the second triplet in the target site;

19 (d) outputting designations and subdesignations of the zinc finger proteins in the
20 first, and second sets identified in step (c).

1 43 A method of producing a zinc finger protein comprising:

2 (a) providing a database comprising:

3 designations for a plurality of zinc finger proteins, each

4 protein comprising at least first, and second fingers;

5 subdesignations for each of the fingers of each of the zinc

6 finger proteins; and
7 a corresponding nucleic acid sequence for each zinc finger
8 protein, each sequence comprising first, and second triplets specifically bound by the first
9 and second fingers respectively, the triplets being arranged in the nucleic acid sequence
10 (3'-5') in the same respective order as the first and second fingers are arranged in the
11 zinc finger protein (N-terminal to C-terminal);

12 (b) providing a target site for design of a zinc finger protein, the
13 target site comprising contiguous first, second and third triplets ordered 3'5' in the target
14 site;

15 (c) for the first and third triplet in the target site, identifying first
16 and second sets of zinc finger protein(s) in the database, the first set comprising zinc
17 finger protein(s) comprising a finger specifically binding to the first triplet in the target
18 site, the second set comprising zinc finger protein comprising a finger specifically
19 binding to the third triplet in the target site;

20 (d) outputting designations and subdesignations of the zinc finger proteins in the
21 first, and second sets identified in step (c).

1 44 A computer program product for selecting a target sequence within
2 a polynucleotide for targeting by a zinc finger protein, comprising:

3 (a) code for providing a polynucleotide sequence;

4 (b) code for selecting a potential target site within the polynucleotide
5 sequence; the potential target site comprising first, second and third triplets of bases at
6 first, second and third positions in the potential target site;

7 (c) code for calculating a score for the potential target site from a
8 combination of subscores for the first, second, and third triplets, the subscores being
9 obtained from a correspondence regime between triplets and triplet position, wherein each
10 triplet has first, second and third corresponding positions, and each corresponding triplet
11 and position has a particular subscore;

12 (d) code for repeating steps (b) and (c) at least once on a further potential
13 target site comprising first, second and third triplets at first, second and third positions of
14 the further potential target site to determine a further score;

15 (e) code for providing output of at least one of the potential target site
16 with its score

17 (f) a computer readable storage medium for holding the codes

1 46. A system for selecting a target sequence within a polynucleotide
2 for targeting by a zinc finger protein, comprising:

3 (a) a memory;

4 (b) a system bus;

5 (c) a processor operatively disposed to:

6 (1) provide or receive a polynucleotide sequence;

7 (2) select a potential target site within the polynucleotide sequence; the
8 potential target site comprising first, second and third triplets of bases at first, second and
9 third positions in the potential target site;

10 (3) calculate a score for the potential target site from a combination of
11 subscores for the first, second, and third triplets, the subscores being obtained from a
12 correspondence regime between triplets and triplet position, wherein each triplet has first,
13 second and third corresponding positions, and each corresponding triplet and position has
14 a particular subscore;

15 (4) repeat steps (2) and (3) at least once on a further potential target site
16 comprising first, second and third triplets at first, second and third positions of the further
17 potential target site to determine a further score;

18 (5) provide output of at least one of the potential target site with its score.

1 47. The system of claim 46, wherein the processor is further operatively
2 disposed to combine a context parameter with a subscore.

1 48. A computer program product for designing a zinc finger protein
2 comprising:

3 (a) code for providing a database comprising
4 designations for a plurality of zinc finger proteins, each protein
5 comprising at least first, second and third fingers ,
6 subdesignations for each of the three fingers of each of the zinc
7 finger proteins;

8 a corresponding nucleic acid sequence for each zinc finger protein,
9 each sequence comprising at least first, second and third triplets specifically bound by the
10 at least first, second and third fingers respectively in each zinc finger protein, the first,
11 second and third triplets being arranged in the nucleic acid sequence (3'-5') in the same
12 respective order as the first, second and third fingers are arranged in the zinc finger
13 protein (N-terminus to C-terminus);

14 (b) code for providing a target site for design of a zinc finger protein, the
15 target site comprising at least first, second and third triplets,

16 (c) for the first, second and third triplet in the target site, code for
17 identifying first, second and third sets of zinc finger protein(s) in the database, the first set
18 comprising zinc finger protein(s) comprising a finger specifically binding to the first
19 triplet in the target site, the second set comprising a finger specifically binding to the
20 second triplet in the target site, the third set comprising a finger specifically binding to the
21 third triplet in the target site;

22 (d) code for outputting designations and subdesignations of the zinc finger
23 proteins in the first, second, and third sets identified in step (c).

24 (e) a compute readable storage medium for holding the codes.

1 49. A system for designing a zinc finger protein comprising:

2 (a) a memory;
3 (b) a system bus;
4 (c) a processor operatively disposed to:
5 (1) provide a database comprising
6 designations for a plurality of zinc finger proteins, each protein comprising
7 at least first, second and third fingers, subdesignations for each of the three fingers of
8 each of the zinc finger proteins;
9 a corresponding nucleic acid sequence for each zinc finger protein, each
10 sequence comprising at least first, second and third triplets specifically bound by the at
11 least first, second and third fingers respectively in each zinc finger protein, the first,
12 second and third triplets being arranged in the nucleic acid sequence (3'-5') in the same
13 respective order as the first, second and third fingers are arranged in the zinc finger
14 protein (N-terminus to C-terminus);
15 (2) provide or be provided with a target site for design of a zinc
16 finger protein, the target site comprising at least first, second and third triplets,
17 (3) for the first, second and third triplet in the target site,
18 identifying first, second and third sets of zinc finger protein(s) in the database, the first set
19 comprising zinc finger protein(s) comprising a finger specifically binding to the first
20 triplet in the target site, the second set comprising a finger specifically binding to the
21 second triplet in the target site, the third set comprising a finger specifically binding to the
22 third triplet in the target site;
23 (4) output designations and subdesignations of the zinc finger
24 proteins in the first, second, and third sets identified in step (3).

1 50. A computer program product for selecting a target site within a
2 target sequence for targeting by a zinc finger protein comprising:

3 code for providing a target nucleic acid to be targeted by a zinc finger
4 protein;

5 code for outputting a target site within the target nucleic acid comprising
6 5'NNx aNy bNzc3', wherein

7 wherein each of (x, a), (y, b) and (z, c) is (N, N) or (G, K);

8 at least one of (x, a), (y, b) and (z, c) is (G, K). and

9 N and K are IUPAC-IUB ambiguity codes;
10 and a computer readable storage medium for holding the codes.

1 51. A system for selecting a target site within a target sequence for
2 targeting by a zinc finger protein comprising:

3 (a) a memory;
4 (b) a system bus;
5 (c) a processor operatively disposed to:
6 provide a target nucleic acid to be targeted by a zinc finger protein;
7 output a target site within the target nucleic acid comprising 5'NNx aNy

8 bNzc3', wherein

9 wherein each of (x, a), (y, b) and (z, c) is (N, N) or (G, K);

10 at least one of (x, a), (y, b) and (z, c) is (G, K) and

11 N and K are IUPAC-IUB ambiguity codes.